

Use of the Sex Ratio as a Means of Resource Assessment for the Japanese Eel *Anguilla japonica*: A Case Study in the Kaoping River, Taiwan

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(Accepted December 1, 2005)

Yu-San Han and Wann-Nian Tzeng (2006) Use of the sex ratio as a means of resource assessment for the Japanese eel *Anguilla japonica*: a case study in the Kaoping River, Taiwan. *Zoological Studies* 45(2): 255-263. A negative relationship between the proportion of females and the population density was found when compiling historical data of both wild and cultured Japanese, European, and American eels. Based on the relationship, the population status of the Japanese eel *Anguilla japonica* was assessed. Japanese eel samples were collected from the lower reaches of the Kaoping River in southwestern Taiwan from 1998 to 2002, and the population density was estimated by mark-recapture experiments in 2001 and 2002. The sex ratio of the eel was skewed towards females, accounting for 81.3%-88.3% in the total samples of yellow and silver eels and for 63.6%-81% in the silver stage eels. The significant female-skewness of the sex ratio validated that the population of Japanese eels in the Kaoping River has declined to a very low level in recent years. The population density of the Japanese eel in the lower reaches of the Kaoping River was approximately 0.01 eels/m² in 2001 and 2002. The decline in the Japanese eel population in the Kaoping River has probably resulted from both overfishing of glass eels in the estuary for aquaculture needs which severely influences recruitment, and degradation of the growth habitat of the yellow eel along the river. The sex ratio therefore is a fast and reliable indicator for eel resource assessment. <http://zoolstud.sinica.edu.tw/Journals/45.2/255.pdf>

Key words: Japanese eel (*Anguilla japonica*), Resource assessment, Sex ratio, Mark-recapture, Population density.

The Japanese eel, *Anguilla japonica* Temminck and Schlegel, is a catadromous fish (Ege 1939), spawning in the waters west of the Mariana Is. (Tsukamoto 1992) and growing in the estuaries and rivers of East Asia (Tesch 1977). After hatching, the eel larvae (leptocephali) drift with the North Equatorial Current and Kuroshio Current from the spawning ground to the continental shelves of East Asian countries and then metamorphose into glass eels which leave the strong oceanic currents and migrate into coastal waters (Cheng and Tzeng 1996, Tzeng 2003). Glass eels become elvers in the estuaries then feed and grow as yellow eels in rivers for 5-8 yrs until metamor-

phosis from yellow to silver eels (Tzeng et al. 2000). After maturation, eels migrate back to their birthplace to spawn and die (Tesch 1977). The Japanese eel is a commercially important cultured species. For cultivation purposes, elvers in the estuary have been overexploited for many years, which has severely influenced the recruitment of the eel and diminished eel populations in the rivers of Taiwan (Tzeng 1984, 1985 Tzeng et al. 1995, Liao 2001, Tzeng 2004).

European (*A. anguilla*) and American (*A. rostrata*) eels have been reported to have ZW sex chromosomes (Passakas and Klekowski 1972, Park and Grimm 1981). However, further kary-

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otype studies failed to identify heteromorphic sex chromosomes (Sola et al. 1980, Wiberg 1983). The eel before the elver stage is considered to be intersexual, and sexual differentiation occurs in the yellow eel stage. Ovaries are found in European eels of 22-30 cm in total length, possibly derived from undifferentiated gonads or from the Syrski organ, a gonad containing both early testes and oogonia. Spermatogonium B-type testes were observed in eels longer than 30 cm (Colombo and Grandi 1996). Sexually undifferentiated elvers can be feminized by treatment with estradiol-17 β (Degani and Kushnirov 1992, Satoh et al. 1992, Chiba et al. 1993), indicating that the sex of the eel is labile. However, the mechanisms of sexual labilization and delayed sexual development in eels are poorly known.

In the wild, eel sex ratios can vary widely, ranging from almost all males to predominantly females (Matsui 1972, Parsons et al. 1977, Tesch 1977, Jessop 1987, Tzeng et al. 1995 2002, Oliveira 1997 1999, Oliveira and McCleave 2000, Oliveira et al. 2001). If the sex determination of the eel is genotype-dependent, then the uneven distributions of sexes in rivers might result from habitat choice, with females preferring to migrate upstream and males preferring to stay in estuaries (Bertin 1956, Tesch 1977), or perhaps females prefer a habitat suitable for faster growth, while males prefer a slow-growth habitat (Helfman et al. 1987). If eel sex determination is phenotype-dependent, then the habitat in which they grow will affect their sex differentiation. Parsons et al. (1977) found that each period of elver restocking into Lough Neagh of the Bann River was followed by a marked increase in the proportion of male silver eels migrating from the lough. Degani and Kushnirov (1992) indicated that 77% of European eels maintained in groups became males, whereas 60% of those maintained in isolation became females. Oliveira et al. (2001) found that the lake area of a river system probably influences the sex ratio of the eel such that eels migrating from lacustrine habitats were mostly females, and eels migrating from fluvial habitats were mostly males. These studies inferred the possible effects of population density on the eel sex ratio. In pond cultures, captivity limits eel migration thus preventing differences in migratory behavior between the sexes. The sex ratio of cultured eels changes with stocking density, and a negative relationship between the proportion of females and stocking density was noted, similar to that found in the wild (Roncarati et al. 1997, Tzeng et al. 2002). Recent

studies favor the environmental sex-determination hypothesis, i.e., at high population densities, males dominate and at low population densities females dominate (Krueger and Oliveira 1999, Tzeng et al. 2002).

Usually, fish stocks are assessed by either laborious mark-recapture experiments or by the time-consuming and fishery-biased data collection system of catch and fishing effort. Finding a simple and reliable way to monitor the resource status would be of great use. Given the hypothesis that the proportion of female eels is negatively related to its population density, 3 major temperate eels *Anguilla anguilla*, *A. japonica*, and *A. rostrata* from both wild and cultured systems were analyzed. The population status of the Japanese eel in a river in Taiwan was assessed by examining the sex ratio. The sex ratio of the Japanese eel collected in the Kaoping River from 1998 to 2002 was analyzed, and the population density was estimated using the mark-recapture method. This adaptive strategy of a labile sex ratio of anguillid eels is also discussed.

MATERIALS AND METHODS

Analysis of the historic sex ratio and population density data

We searched MEDLINE and EMBASE from 1970 to 2004 to acquire sex ratio and population density data of anguillid eels. The search used combined key words like eel with sex ratio, population density, and sex determination. We also searched specialist books and conference proceedings we had on hand regardless of the language. Data of the eel included 4 studies on the Japanese eel, 5 studies on the American eel, and 3 studies on the European eel (Appendix I). Data on the population density of the eel with continuous variables were defined as quantitative and were used for analysis of relationship between the proportion of females and population density. For Japanese eels, all data were included for analysis. For American eels, only the studies of (Oliveira 1997) and (Oliveira and McCleave 2000) were used. For European eels, data from (Roncarati et al. 1997) were used.

Field investigations of the sex ratio

The sex ratio of the Japanese eels caught with eel traps in the lower reaches of Kaoping

River in southwestern Taiwan ($120^{\circ}50'E$, $22^{\circ}40'N$) were recorded approximately every 2 mo during the period from Nov. 1998 to Nov. 2002. Total length (TL, ± 1 mm) and body weight (BW, ± 0.1 g)

of the eels were measured. The sex and developmental stage of each eel was determined by body coloration and gonadal histology when possible (Han et al. 2002).

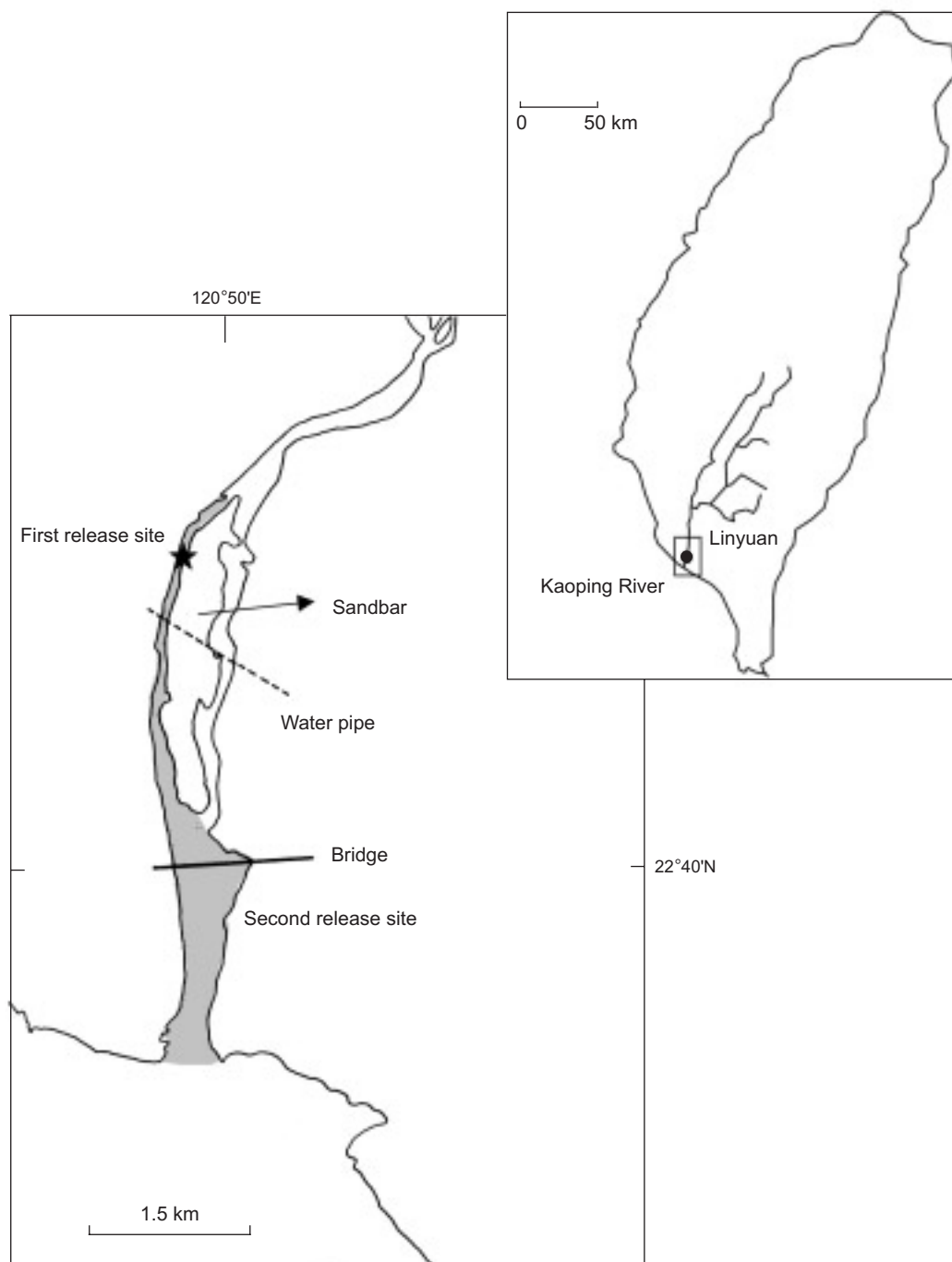


Fig. 1. Sampling site for wild Japanese eels in the lower reaches of the Kaoping River. The asterisk and shaded region indicate the 1st and 2nd release sites, respectively. The area of the shaded region was approximately 1.2 km^2 as estimated by a 1: 10000 scale map copied onto paper with a square grid. Multiplying the squared unit area by the estimated number of squares covering the shaded region gave the area.

The mark-recapture experiment in 2001

In total, 277 eels collected from a culture pond at Lukang in Aug. 2001 were used for a mark-recapture experiment. After anesthetization with 2-phenoxyethanol, microchips were implanted intramuscularly behind the anus of the eels so that they could be detected using an external scanner. The tagged eels were released into the estuary of the Kaoping River on 29 Aug. 2001 (Fig. 1). Captured eels caught by traps within the shaded region of the estuary shown in figure 1 were recorded from 11 Sept. - 29 Nov. 2001. In total, 360 eels were caught and scanned to identify tagged eels.

The mark-recapture experiment in 2002

To eliminate a possible bias in the distributional behavior of cultured eels, 180 eels caught within the shaded region of the Kaoping River estuary shown in figure 1 in June 2002 were used for a tagging experiment. After anesthetization with 2-phenoxyethanol, visible plastic streamer tags were attached to the dorsal fin of the eels, and the eels were then released back into the estuary of the Kaoping River on 11 June 2002 (Fig. 1). In total, 150 eels were caught in the same area from 11 to 25 July 2002, and the tagged eels were identified.

Estimation of population density

The population size of wild Japanese eels in the Kaoping River was estimated by the mark-recapture method of Chapman (Seber 1982) which uses the following equations:

$$N=(M+1)(C+1)/(R+1)-1, \quad (1)$$

$$V(N)=(M+1)(C+1)(M-R)(C-R)/(R+1)^2(R+2), \text{ and } (2)$$

$$95\%CI=N \pm Z_{\alpha}x\sqrt{V(N)}; \quad (3)$$

where N is the population size, M is the number of the marked eels, C is the number of eels caught, R is the number recaptured, $V(N)$ is variance of N , CI is the confidence interval, and Z_{α} is 1.96 with $\alpha = 0.05$. Because of the small size of the recapture sample, Poisson confidence intervals were also introduced (Clopper and Pearson 1934). The exact Poisson confidence intervals for samples were computed using a website tool (available at <http://members.aol.com/johnp71/confint.html#Poisson>). The population density (d) of

eels in the river was estimated by the formula:

$$d = N/A; \quad (4)$$

where N is the population size and A is the area investigated.

Data analysis

Statistical differences in the sex ratios of wild eels among years were examined using the Chi-square test of homogeneity, and differences in the mean TL (\pm SD) and BW (\pm SD) between sexes were examined using Student's t -test. A regression between the proportion of females and density was calculated using the program SigmaPlot 2001.

RESULTS

Relationship between the eel sex ratio and population density

Historic quantitative data on the sex ratio and population density of the Japanese, American, and European eels all showed a similar trend of the proportion of females being significantly negatively correlated to the total population density (Fig. 2). The relationship in the American eel showed no significance when all data were included ($R^2 = 0.14$, $p = 0.09$), however, the relationship turned out to be strongly significant when 2 extreme data points (may be due to some measurement bias) were excluded from the analysis ($R^2 = 0.49$, $p = 0.0005$, Fig 2b). In other words, the eel population was dominated by females when the population density was lower and dominated by males when the population density was higher irrespective of eel species.

Comparison of the length (TL) and weight (BW) between sexes

There were no significant differences in either mean TL ($p = 0.61$) or BW ($p = 0.96$) between females (499.0 ± 4.8 mm, 198.2 ± 7.5 g) and males (505.3 ± 10.4 mm, 197.3 ± 12.9 g) regardless of the developmental stage. The ranges of the TL and BW were wider for females (TL: 271-794 mm; BW: 25.8-851.6 g) than for males (TL: 315-675 mm; BW: 37.7-468.7 g) (Fig. 3a). However, mean values of TL and BW of silver eels were significantly larger ($p = 0.0002$ for both) for females (642.9 ± 10.3 mm, 457.3 ± 25.8 g) than

males (563.9 ± 17.9 mm, 275.5 ± 26.6 g). Silver eels larger than 70 cm TL were all females, while silver ones less than 45 cm TL were all males (Fig. 3b)

Sex ratio

From 1998 through 2002, the sex of 569 Japanese eels collected in the Kaoping River was

determined (Table 1). The proportions of female eels in the river ranged from 81.3% to 88.3% with a mean of 85.2% and did not differ significantly among years ($\chi^2 = 9.764 < \chi^2_{(4, 0.025)} = 11.143$). Silver eels were also predominantly females, ranging from 63.6% to 81.0% with a mean of 73.1% (data not shown), and the proportions did not significantly differ among years ($\chi^2 = 1.298 < \chi^2_{(3, 0.025)} = 9.348$).

Population density

In 2001, totally 360 eels with a mean total length of 527.1 ± 39.8 mm and a mean body weight of 222.7 ± 58.8 g were captured after the

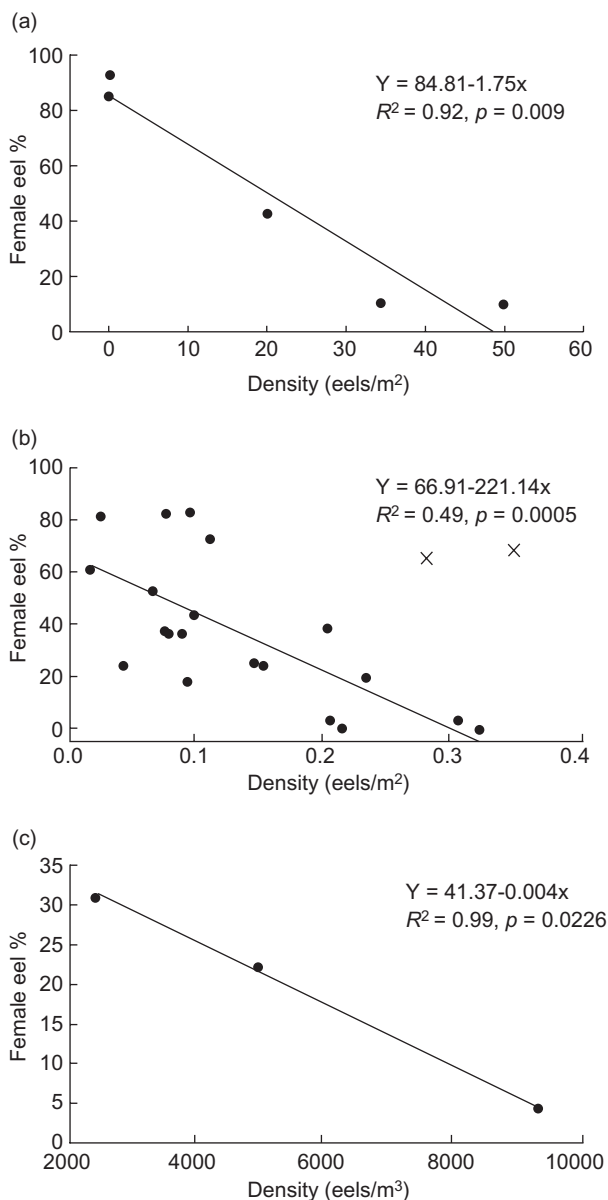


Fig. 2. Relationship between the eel sex ratio and population density in Japanese (a), American (b), and European (c) eels. The density units for Japanese and American eels are eels/m² and for European eel are eels/m³, respectively. The regression equation in (b) was determined by omitting 2 extreme data points (cross). Studies used for analysis of the relationship are shown in the Appendix I.

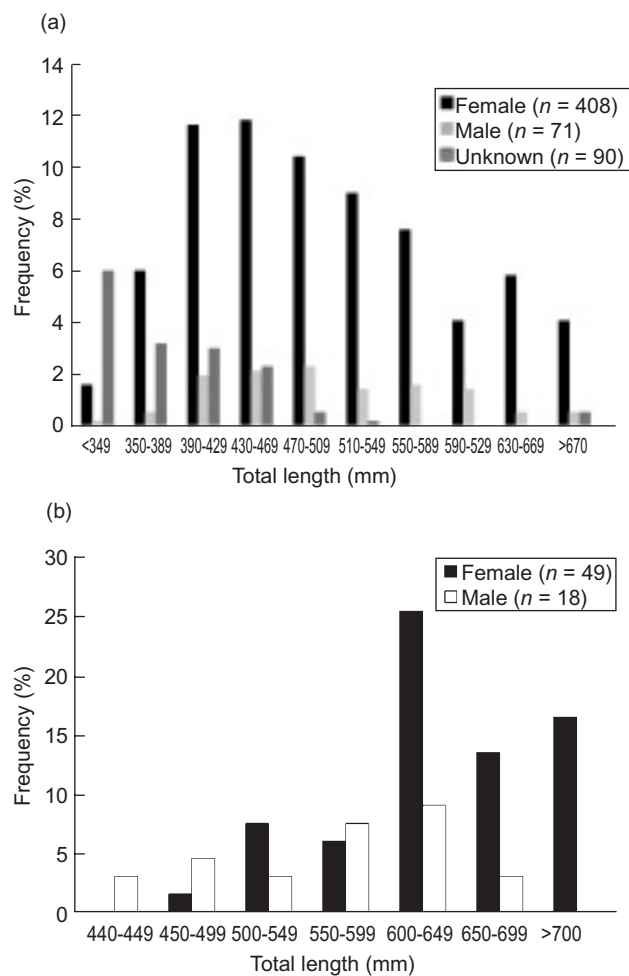


Fig. 3. (a) Frequency distribution of the total lengths of females, males, and gender-undetermined Japanese eels in the Kaoping River. (b) Frequency distribution of the total lengths of silver female and male Japanese eels in the Kaoping River.

release. Among them, 6 tagged eels were recaptured. The recapture rate was 1.7% (Table 2). In 2002, totally 150 eels with a mean total length of 447.2 ± 52.7 mm and a mean body weight of 126.9 ± 60.0 g were captured after the release. Among them, 2 tagged eels were recaptured. The recapture rate was 1.3% (Table 2). The population size of wild Japanese eels in the estuary of the Kaoping River was estimated to be approximately $14,335 \pm 9713$ (95% CI) in 2001 and 9109 ± 8765 (95% CI) in 2002 using Chapman's method (Table 2). Since the population size estimation may have a large bias when fewer than 7 tagged eels are recaptured and the recapture rate is $< 10\%$, Poisson confidence limits were introduced for comparison. The population size ranged 7137-31,360 in 2001 and 3324-22,040 in 2002 using the Poisson confidence intervals with 95% CI (Table 2). As indicated, the ranges of eel population sizes estimated by both Chapman's method and the Poisson confidence intervals were similar. The population density was estimated to be approximately 0.01 eels/m² in both years.

DISCUSSION

The mean TL and BW of both sexes showed

no significant differences ($p > 0.05$) when all eel phases were included, but were significantly larger for silver females than for silver males ($p < 0.05$). This is due to the wider TL and BW ranges of females. Since development of the male gonads occurs later than that of females (Colombo and Grandi 1996), yellow males were sometimes difficult to identify. Thus, some of them may have been grouped with eels of undetermined sex, thus increasing the mean TL and BW of the males but reducing their proportion. This may explain the higher mean proportion of females when all phases were included (85.2%) (Table 1) compared with only silver phase eels (73.1%). Although the ratio of total males may have been underestimated, the sex ratio should be close to the true value when only silver phase eels, which all have developed gonads, were considered.

In today's world of pollution and the prevalence of endocrine-disrupting chemicals, environmentally induced changes in sex ratios are possible. Thus, we analyzed the sex ratio of a goby fish (*Glossogobius aureus*) in the estuary of the Kaoping River for comparison. Out of 136 fish examined, there were 78 males and 58 females. Somewhat more males were found in this case. Therefore, the possible effects of environmental estrogen-like hormones on eel sex determination could be excluded.

Table 1. Sex ratios by year of wild Japanese eels in the Kaoping River

Year	n	No. of eels		Sex undetermined	Female (%) ^a
		Female	Male		
1998	21	16	3	2	84.2
1999	158	106	14	38	88.3
2000	57	46	8	3	85.2
2001	185	136	22	27	86.1
2002	148	104	24	20	81.3
Total	569	408	71	90	85.2

^aSex-undetermined eels were omitted from the calculation of percent of females.

Table 2. Population size of the Japanese eel in the Kaoping River estimated by Chapman's mark-recapture method

Sampling date	No. of eels				Chapman's CI (95%)	Poisson CI (95%)
	Marked	Catch	Recaptured	Population		
Sept.-Nov. 2001	277	360	6	14,335	4622-24,048	7137-31,360
July 2002	180	150	2	9109	344-18,474	3324-22,040

In this study, the number of recaptured eels with tags relative to the total number recaptured was low (Table 2). This may have led to a large bias in the population density estimation. Despite this, the estimated population density still remains quite low even if the abundance estimates are off by an order of magnitude. According to conversations with local fishermen who have been catching eels for more than 30 yrs in the Kaoping River, the eel resources were once abundant (pers. comm.). They said that the eel harvest was several dozen-fold greater 20 yrs ago than it is now with a similar fishing effort, and most eels were males when the resource was abundant. Since more than 80% of Japanese eels are concentrated in the lower reaches (unpubl. data), we can speculate that a sex ratio reversal seems to have occurred for the Japanese eel, and the eel resources in the Kaoping River are endangered. A previous investigation of Japanese eel resources in the Tanshui River of northern Taiwan, which has experienced similar fishery and habitat pressures (Tzeng et al. 1995), also showed a very low population density, and thus may also be experiencing similar short-ages in eel resources.

The negative relationship between the female sex ratio and population density seems to be a common phenomenon among eel species (Fig. 2). The population density was proposed as being a key factor in determining the sex of eels, with habitats having high densities of eels dominated by males and habitats with low densities dominated by females (Colombo and Rossi 1978). Sex differentiation of the eel is probably an adaptive strategy to achieve maximum fitness in which male eels exhibit a time-minimizing growth strategy by maturing as soon as possible, while females postpone maturation with a size-maximizing growth strategy to attain higher fecundity (Vøllestad and Johnson 1986, Helfman et al. 1987, Larsson et al. 1990, Vøllestad 1992). In high-density habitats, food resources are limited, and the earlier maturation of males may promote an earlier spawning migration. This possibly reduces intraspecific competition and the mortality rate of the eel. In contrast, in low-density stocks, food resources are relatively plentiful and eels differentiating into females would have enough time to fully utilize the resources and grow larger to achieve higher fecundity and increased reproductive success (Tzeng et al. 2000 2002). Furthermore, although the sex ratios of the eels in different habitats may be highly variable, the final sex ratio of the entire spawning population would be suitable for maximum population growth when

they migrate back to the spawning ground.

In Taiwan, Japanese eel elvers in the estuary have been overexploited for aquaculture, and the adult eels in the rivers have also been over-fished due to their high price (Tzeng 1984 1997). These factors have probably led to both population declines and the skewed sex ratios. In Japan, the catches of both glass eels and adult eels have also shown decreasing tendencies (Tatsukawa 2003). If Japanese eel resources severely decline throughout East Asia, it might result in a widespread sex ratio bias and catastrophic population collapse. Thus, to sustain natural eel resources, habitat protection and prevention of over-fishing are urgently needed.

Giving the unique character of the eel sex ratio in relationship to population density, long-term changes in the eel sex ratio may reflect long-term changes in the eel resource. In conclusion, the use of the sex ratio as an indicator of the eel resource status may be helpful for eel conservation. An analysis of the latest 5 yrs of eel sampling found that the sex ratio was strongly skewed towards females, which contrasts with historic information, suggesting imminent danger to the eel resource in the Kaoping River. The hypothesis that the variation in sex ratio in conjunction with different growth strategies for each sex serves to maximize the adaptive fitness of the eel might turn out to be hazardous when eel sex ratios in all habitats significantly shift toward females.

Acknowledgments: This study was financially supported by the Council of Agriculture, Executive Yuan, Taiwan, R.O.C. (90AS-1.4.5-FA-F1-36 and 91AS-2.5.1-FA-F1-8). The authors are grateful to Mr. G. H. Cheng for sample collection and data processing and Mr. B. M. Jessop of Canada for reviewing an early draft of the manuscript.

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APPENDIX I : Studies used for analysis of the relationship between the eel sex ratio and population density

Study	Species ^a	<i>n</i>	Habitat	Density character
This study	AJ	479	Wild	Eels/m ²
Tzeng et al. 1995	AJ	97	Wild	Eels/m ²
Sato and Nimura 1991 1992	AJ	84	Cultured	Eels/m ²
Tzeng et al. 2002	AJ	49	Cultured	Eels/m ²
Tzeng et al. 2002	AJ	40	Cultured	Eels/m ²
Jessop 1987	AR	635	Wild	Lake area/Drainage area
Oliveira 1997	AR	245	Wild	Eels/m ²
Oliveira 1999	AR	1718	Wild	Lake area/Drainage area
Oliveira and McCleave 2000	AR	1098	Wild	Eels/m ²
Oliveira et al. 2001	AR	3273	Wild	Lake area/Drainage area
Parsons et al. 1977	AA	10346	Wild	Elver restocking
Degani and Kushnirov 1992	AA	18	Cultured	Isolated/Grouped
Roncarati et al. 1997	AA	212622	Cultured	Eels/m ³

^aAJ: *Anguilla japonica*; AR: *A. rostrata*; AA: *A. anguilla*.